**Original Article**

**Effects of in vivo exposure to eggs with sperm-immobilizing antibodies in follicular fluid on subsequent fertilization and embryo development in vitro**

HIROAKI SHIBAHARA,* YUKI HIRANO, YASUKO SHIRAISHI, KAZUHIKO SHIMADA, KUMIKO KIKUCHI, TATSUYA SUZUKI, SATORU TAKAMIZAWA and MITSUAKI SUZUKI

Department of Obstetrics and Gynecology, School of Medicine, Jichi Medical University, Tochigi, Japan

**Aims:** It has been shown that supplementation of patients’ sera that contains sperm-immobilizing antibodies results in failure of fertilization and embryo development *in vitro*. The present study was carried out to investigate if exposing retrieved eggs to a high number of sperm-immobilizing antibodies in the follicular fluid (FF) *in vivo* affected subsequent fertilization and embryo development *in vitro*, even if they were washed with an antibody-free culture medium.

**Methods:** Patients’ sera and their FF were collected in 15 *in vitro* fertilization-embryo transfer (IVF-ET) or intracytoplasmic sperm injection-embryo transfer (ICSI-ET) treatment cycles from 11 infertile women with sperm-immobilizing antibodies in their sera. Quantitative sperm-immobilizing antibody titers (SI50 titers) in the sera and FF were evaluated. The fertilization rate, good-quality embryo rate and implantation rate by IVF-ET were compared between infertile patients having higher (10 ≤) SI50 titers and lower (<10) SI50 titers in their FF.

**Results:** There was a significant correlation in the SI50 titers between the patients’ sera and their FF (P < 0.0001). After thoroughly washing the collected eggs in culture medium without the patient’s serum before IVF, there was no difference in the fertilization rate in the patients with high (10≤) and low (<10) SI50 titers in their FF (P = 0.62). However, the good-quality embryo rate in the patients with a high SI50 titer was significantly lower than patients with a low antibody titer (P < 0.05). There was no significant difference in the implantation rate between the two groups (P = 0.33).

**Conclusions:** Similar amounts of sperm-immobilizing antibodies existed in the patients’ FF and in their sera. ICSI did not seem to be necessary in patients having the antibodies if their sera were not supplemented in the culture media. Even with careful manipulation of eggs, it might be suggested that the harmful effects of sperm-immobilizing antibodies on embryo development cannot be completely avoided, especially in patients with high SI50 titers in the FF. (Reprod Med Biol 2006; 5: 137–143)

**Key words:** antisperm antibody, embryo development, female infertility, follicular fluid, *in vitro* fertilization, sperm-immobilizing antibody.

---

**INTRODUCTION**

Immunoological causes of infertility, such as antisperm antibodies in both males and females, are among the most frustrating pathologies for conventional treatments with timed intercourse or intrauterine insemination (IUI). The incidence of infertile women having sperm-immobilizing antibodies, detected by the sperm immobilization test (SIT), in their sera has been shown to be approximately 3% when it is tested for in female patients first visiting infertility outpatient clinics. The presence of antibodies against sperm, especially sperm-immobilizing antibodies, in the sera of infertile women has been shown to inhibit sperm migration in the female genital tract, including cervical mucus and the fallopian tubes. The antibodies result in poor postcoital testing or refractory response to the treatments with IUI. However, some infertile women who have sperm-immobilizing antibodies in their sera establish pregnancy without the use of *in vitro* fertilization-embryo transfer (IVF-ET) treatment when the antibody titers are relatively low at the time of conception. Kobayashi et al. found that the antibody titers evaluated by a quantitative SIT (SI50; the 50% sperm immobilization...
units), as described by Isojima et al., correlated to possible conception by IUI. They concluded that infertile women with SI<sub>50</sub> titers lower than 10 could conceive by IUI, whereas those with antibody titers higher than 10 could not. Thereafter, the cut-off value of 10 for SI<sub>50</sub> titers was used when a clinical decision for treatment was made.

Such antibodies can also exert inhibitory effects on various stages of sperm–egg interaction and subsequent embryo development in vitro. To avoid low fertilization rates and poor embryo quality in vitro, it is recommended that the manipulation of gametes and embryos from patients having sperm-immobilizing antibodies should be carried out more carefully than usual. Thorough washing of eggs collected in the culture medium to prohibit contamination with the patient’s serum and follicular fluid (FF) should be carried out in order to obtain a better IVF result. However, it has not yet been clarified whether high titers of sperm-immobilizing antibody in FF affect fertilization and embryo development in vitro, even when the eggs are treated as mentioned above. If fertilization is inhibited by the existence of a higher number of sperm-immobilizing antibodies, intracytoplasmic sperm injection (ICSI) should be theoretically carried out.

The present study was carried out to investigate if exposing the retrieved eggs to a high number of sperm-immobilizing antibodies in the follicular fluid (FF) affected subsequent fertilization and embryo development in vitro, even if they were washed with the antibody-free culture medium.

**MATERIALS AND METHODS**

**Patients’ sera**

A total of 1020 infertile women between May 1999 and December 2004 at the Department of Obstetrics and Gynecology, School of Medicine, Jichi Medical University, Japan, were tested for sperm-immobilizing antibodies in their sera as described below. Twenty-seven of 1020 infertile women had sperm-immobilizing antibodies in their sera, giving the positive rate of 2.6%. So far, 11 of them proceeded to IVF-ET or ICSI-ET treatment after the unsuccessful treatment cycles by intrauterine insemination. The average age was 37.7 years (range 26–44 years). The treatment by ICSI was selected for two infertile women with sperm-immobilizing antibodies because of their husband’s severe asthenozoospermia.

Patients’ sera were collected in 11 IVF-ET or four ICSI-ET treatment cycles from 11 infertile women having sperm-immobilizing antibodies in their sera, with informed consent. All sera were heat-treated at 56°C for 30 min to inactivate them and were kept frozen at −20°C until use.

**Collection of follicular fluid and procedure of IVF-ET and ICSI-ET**

A standardized ovarian stimulation was carried out, as previously described. The patients were stimulated using a gonadotropin releasing hormone (GnRH) agonist (Nafarelin acetate, Yamanouchi Pharmaceutical, Tokyo, Japan) started in the midluteal phase (suppression protocol) followed by gonadotropins. After the onset of withdrawal bleeding, ovarian stimulation was initiated by injections of follicle stimulating hormone (FSH; Fertinom P, Serono, Tokyo, Japan) for 3 days followed by human menopausal gonadotropins (Humegon, Nippon Organon K.K., Osaka, Japan) for >6 days. Ovarian stimulation was monitored by the measurement of serum E<sub>2</sub> concentration and by ultrasonographic assessment of the follicle diameter. Human chorionic gonadotropin (hCG) (HCG, Mochida, Tokyo, Japan) was injected when at least one of the leading follicles reached 17 mm in diameter. Oocyte retrieval was carried out through transvaginal ultrasonography-guided aspiration 36 h after the hCG administration. Retrieved oocytes were examined under a microscope and washed with culture media supplemented with human serum albumin (HSA) at least three times before pre-incubation. Before insemination with the husband’s swim-up sperm, they were washed again with new culture media four times.

The FF from the leading follicle was collected in 11 IVF-ET or four ICSI-ET treatment cycles from 11 infertile women having sperm-immobilizing antibodies in their sera, with informed consent. All FF were immediately centrifuged for 5 min at 600 × g and the supernatants were heat-treated at 56°C for 30 min for inactivation. They were kept frozen at −20°C until use.

On the second or third day after IVF or ICSI, the morphological assessment of embryos was carried out under an inverted microscope using the Veeck’s classification before transfer. Grade 1 embryos with regular blastomeres and no cytoplasmic fragments, and grade 2 embryos with regular blastomeres and minor cytoplasmic fragments were considered good-quality embryos. Grade 3, 4 and 5 embryos were considered poor-quality embryos. A maximum of three embryos of good quality were transferred. In some selected cases, elective transfer of two good-quality embryos was carried